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Effect of Low-Level Laser Therapy (LLLT) on Viscoelasticity of the Contracted Knee Joint: Comparison With Whirlpool Treatment in Rats

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Background and Objective: **The purpose of this study was to compare the effect of Low-Level Laser Therapy (LLLT) with sham and whirlpool treatment on the contracted knee joint in rat.**
Study Design/Materials and Methods: **Forty-eight Wistar rats were operated on to immobilize knee joint, and 1 week after operation they were randomly assigned to four treatment groups: laser 40 mW (3.9 W/cm^2), laser 60 mW (5.8 W/cm^2), whirlpool (42°C), and sham laser. Tunable Ga-Al-As semiconductor (810 nm) laser was used for another 2 weeks of treatment. Removing and preparing bilateral hind legs, degree of knee contracture was assessed by measuring the knee flexion angle, weight of the gastrocnemius muscle, and periarticular connective tissue viscoelasticity measuring phase-lag and stiffness.**
Results and Conclusion: **Laser irradiation showed no significant changes except the phase-lag of laser 60 mW. Under the conditions of this study, LLLT stimulation did not provide a significant effect for minimizing the degree of experimental joint contracture over whirlpool treatment. Lasers Surg. Med. 22:81–85, 1998.**

Key words: **animal experiment; joint immobilization; laser biostimulation; viscoelastic measurement; whirlpool treatment**

INTRODUCTION

Joint contracture is a consequence of immobilization or various traumas and is one of the most prevalent syndromes encountered in physical therapy. Joint contracture resulting from prolonged bed rest or immobilization leads to a greater degree of disability and jeopardizes the rehabilitation process. Although high-energy laser therapy for surgical cutting and hemostasis is well known, the effect of low-energy laser, which usually means <100 mW power intensity and is regarded as showing a nonthermal effect, still remains surrounded by skepticism and controversies over investigated results [1]. Low-Level Laser Therapy (LLLT) has recently been studied for modulating various processes in biological systems [2–4], e.g., tissue healing, mainly cutaneous wound [5,6], pain control and nerve function [7–9], and arthritis and other lesions [10–13].

The purpose of this *in vivo* study was to compare the effect of the low-level laser with conventional whirlpool treatment applied to the contracted knee joint. The outcome in animal models was assessed in view of viscoelastic properties of

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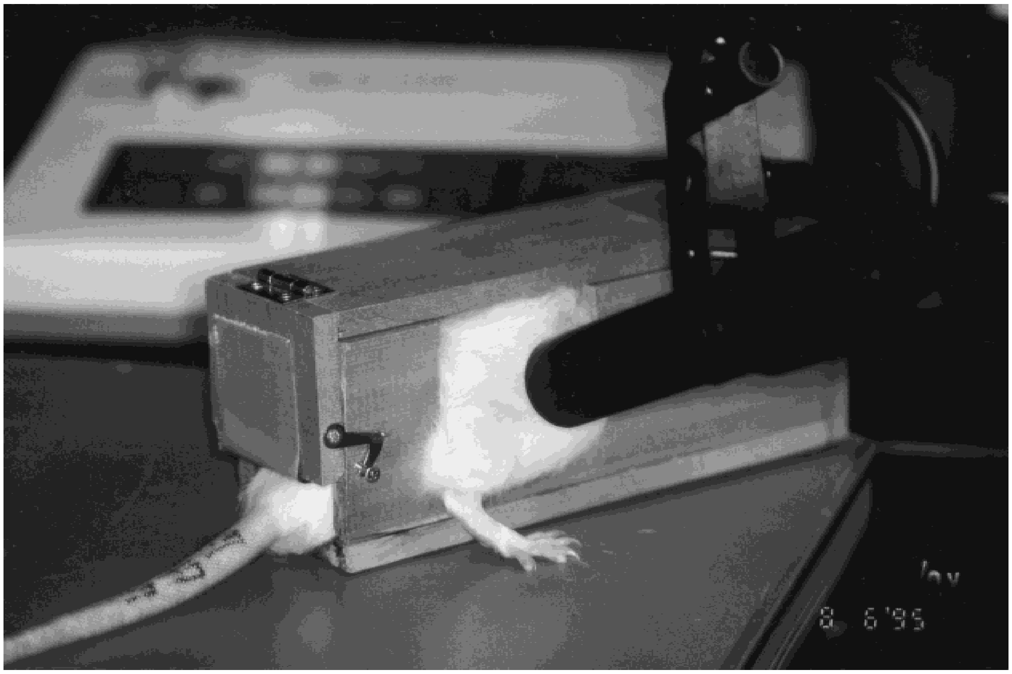

Fig. 1. Photograph of laser treatment. The animal was held in a box and restrained during each treatment.

joint tissue and gastrocnemius muscle weight as a first step in understanding the possible role of laser biostimulation in the musculoskeletal system.

MATERIALS AND METHODS

Animals and Operative Procedure

Forty-eight 9-week old male Wistar-Imamichi rats, initially weighing 300–350 g, were used. Under intraperitoneal anesthesia (40 mg/kg pentobarbital sodium), subcutaneous femorotibial ligature [14] with nonabsorbable suture on the right extremity was used to keep the knee joint in ~150° of flexion. A needle without a suture was passed on the left extremity as a control limb. All rats were kept freely for 1 week after operation and then randomly assigned to four groups of ① laser 40mW, ② laser 60mW, ③ whirlpool (WP), and ④ sham laser.

Treatment Protocol and Equipment

Treatment protocol was planned on the basis of a preliminary study with the same materials using 10 mW (irradiance 0.6 W/cm^2) and cumulative doses 15 and 45 J. Because no significant difference was noted between the animals restrained in a box with sham laser treatment and free animals that were not restrained in a box, we used sham laser animals as a control group in this experiment. All animals received a total of six treatments: three treatments a week (every 2 days on weekdays) for another 2-week period given by one of us (M.U.).

Lasers 40 mW and 60 mW. The laser (JQ305, Nihon Infrared Industry, Kawasaki, Japan) used was a Ga-Al-As semiconductor type, wavelength 810 nm, tuneable intensity up to 100 mW, continuous beam. Output was set at 40 mW and at 60 mW. The beam geometry was rectangular and the spot area was 1.04 mm^2. During laser treatment, the animals were kept in a special wooden box and were unable to stir. The tip of the laser probe was attached directly to the lateral joint space of the immobilized knee (experimental limb) through an open window of the box (Fig. 1). As this device was unable to operate continuously for >1 minute, the procedure was consequently repeated three times for 3 minutes. The total dose per treatment was 7.2 J ($= 3 \times 60 \times 40/1{,}000$) for 40 mW, and 10.8 J ($=3 \times 60 \times 60/1{,}000$) for 60 mW, and the accumulated dose for all six treatments was 43.2 J and 64.8 J, and the incident energy density was 3.85 W/cm^2 and 5.77 W/cm^2, respectively.

Whirlpool. The tub used (Racom Ace HT-100V, Iuchi, Japan) was 25 × 39 × 26 cm and water temperature was automatically maintained at 42°C. The rats were forced to swim for 7 minutes in the bath with the help of a chest harness.

Sham laser. The rats in this group did not receive any active treatment, although they did receive the same procedure in the box as the laser groups.

Measurement of Muscle Weight and Preparation for Specimen

On the 21st day after operation, the rats were killed with an overdose of anesthetic ether and both hind legs were disarticulated at the hip. The gastrocnemius muscle was carefully removed and weighed with an electronic scale (JL-180, Chyo Balance Corp., Kyoto, Japan). The other muscles of the thigh and lower leg were removed, leaving the joint capsule and ligaments of the knee intact. After disarticulation through the ankle, each specimen was wrapped with wet gauze and stored in a plastic bag at −80°C. The specimens were thawed at room temperature just before knee flexion angle (KFA) measurement and viscoelastic analysis.

Measurement of Knee Flexion Angle

With the knee joint in maximum extension, the femoral shaft was fixed in a holding device to keep the lower leg in a horizontal position. After the addition of 0.02 N weight (the averaged weight of the foot known from a preliminary study; 1.98 ± 0.12 g) at the distal end of lower leg, the KFA, the external angle between the long axis of the femur and the tibia, was measured using a plastic goniometer.

Measurement of Viscoelasticity

Forced vibration method utilized spectral analysis to determine how mechanical signals were modified in amplitude and phase as they pass through the knee joint [15]. Spectral analyzer (3582A, Hewlett Packard, Corvallis, OR) worked as a dual channel Fast Fourier Transform machine and provided electrical driving signals to the viscoelastic spectrometer (DDV-VMF, Orientec Co., Yokohama, Japan), which were converted into random mechanical noise with a frequency of 1–50 Hz. The transfer functions, the mathematical relationship between both input and output signals through the knee joint, indicated a delay of phase (phase lag) and a structural stiffness in each specimen. These were shown on the display of the analyzer and recorded on the X-Y plotter (D-73BP, Riken Densi, Tokyo, Japan). Reading of values of phase lag and stiffness at frequency of 5, 15, 25, and 35 Hz were selected for convenience.

Statistical Analysis

Statistical analyses according to analysis of variance (ANOVA) were applied to test for differences among the four groups for body weight gain, gastrocnemius muscle weight, KFA, phase lag, and stiffness. The Student's *t*-test also was used to compare the experimental and control limbs. The analysis was carried out using a commercially available computer software program (Statview 4.5, Abacus Concepts, Berkeley, CA).

RESULTS

Status of the Animals

Of the original 48 rats entered in this study, 12 were eliminated due to loosened suture (one), to little weight gain with or without infectious abscess at the dissection (eight), and to an undetectable graph of X-Y plotter in viscoelasticity measurement (three). Among the available 36 rats for the analysis, no significant differences were found in body weight gain during the 3 weeks, i.e., 10 for laser 40 mW (70.7 ± 10.2g), nine for laser 60 mW (75.6 ± 16.7g), nine for whirlpool (83.1 ± 19.6g), and eight for the sham laser (76.8 ± 16.6g).

Gastrocnemius Muscle Weight and Knee Flexion Angle

The muscle weight of the experimental limb was significantly less than that of the control limb, indicating the presence of muscular atrophy of the experimental limb in all groups. The WP group showed significantly heavier muscle weight in both limbs than the other groups. However, the ratio between the experimental limb and control limb failed to show any difference among the all groups (Table 1). The KFA of the experimental limb was significantly larger than that of the control limb, indicating presence of flexion contracture by immobilization. Among the groups, the only significant result was in the WP group, where the KFA had decreased more than in other groups.

Viscoelastic Measurement

In this experimental condition, the stiffness (elastic resistance against extension load) varied widely and failed to show differences among the groups. Phase-lag (viscous nature as shock-absorbing property) of the experimental limb was significantly less than that of the control limb, i.e., development of joint contracture. Among the

TABLE 1. Weight of Gastrocnemius Muscle and Knee Flexion Angle†

Groups	Muscle weight (g) Control limb	Muscle weight (g) Experimental limb		Knee flexion angle (degrees) Control limb	Knee flexion angle (degrees) Experimental limb
Laser 40 mW	2.61 ± 0.15	2.41 ± 0.12	(91.2%)	64 ± 12	104 ± 7
Laser 60 mW	2.44 ± 0.12	2.24 ± 0.13	(91.9%)	64 ± 7	106 ± 6
Whirlpool	2.74 ± 0.27*[1]	2.44 ± 0.16*[2]	(90.1%)	58 ± 5	95 ± 7*[3]
Sham laser	2.40 ± 0.09	2.09 ± 0.26	(89.8%)	57 ± 5	105 ± 3

†mean ± standard deviation; values in parenthesis indicate the ratio between experimental limb and control limb (%).
*P values significantly different from the sham laser among group comparison: *[1]$P = 0.0008$, *[2]$P = 0.0256$, *[3]$P = 0.0050$.

TABLE 2. Phase-lag With Viscoelastic Measurement (degrees)†

Frequency (Hz)	Control limb Sham laser	Experimental limb Laser 40 mW	Laser 60 mW	Whirlpool	Sham laser
5	50 ± 4*[1]	21 ± 8	40 ± 13*[2]	38 ± 10*[3]	28 ± 8
15	41 ± 3*[1]	15 ± 7*[4]	30 ± 10	30 ± 7*[5]	23 ± 7
25	28 ± 4*[1]	9 ± 6	19 ± 8	19 ± 7*[6]	12 ± 7
35	13 ± 4*[7]	4 ± 4	8 ± 4	8 ± 5	3 ± 4

†mean ± standard deviation.
*P values significantly different from the sham laser group of experimental limb at the same frequency. *[1]$P < 0.0001$, *[2]$P = 0.0061$, *[3]$P = 0.0400$, *[4]$P = 0.0451$, *[5]$P = 0.0491$, *[6]$P = 0.0497$, *[7]$P = 0.0002$.

groups, the phase-lag showed significantly higher values in WP and laser 60 mW groups, especially at lower frequency over the sham laser, whereas it had a reverse lower value in laser 40 mW at 15 Hz (Table 2).

DISCUSSION

Although several clinical [10–13,16] and *in vitro* [17] studies have failed to demonstrate a significant effect, a few experimental studies still showed that LLLT increases collagen synthesis [18] and enhances skeletal muscle regeneration [19] through cellular function change. Local heat, such as whirlpool treatment or ultrasound, produces some extensibility of collagen tissues and alters the material properties of fibrous tissues [20,21].

If laser irradiation had an effect on minimizing joint contracture caused by shrinking periarticular fibrous tissues and by changing tissue remodeling, the expected viscoelastic changes in this experiment would indicate high phase-lag and low stiffness. In this experimental protocol, the gastrocnemius muscle as material was far away from the direct influence of the laser beam.

In the present study, only whirlpool treatment showed significant effectiveness in all variables of muscle weight, KFA, and phase-lag. The retaining muscle weight in both limbs might be due to increased blood circulation from local heat and general exercise. Both laser treatments had no effect on maintaining muscle weight. Laser 60 mW had the same high value of phase-lag at 5 Hz as in whirlpool treatment, but failed to decrease KFA compared to sham laser. Laser 40 mW had no effect on KFA and even had decreased phase-lag reversibly.

The stimulating doses in this experiment were 3.9–5.8 W/cm^2 and were regarded as non-thermal biostimulation. Therefore, the low-level laser irradiation, not in the form of heat but in the form of photochemistry in nature, may affect soft tissue remodeling to some extent, but its effect was less obvious than the thermal one by whirlpool. This was partly compatible with the previous study by Weiss and Oron [19] indicating the more rapid regeneration process in the laser irradiated gastrocnemius muscle.

In view of minimizing joint contracture, there was no obvious advantage in using laser treatment compared to the conservative whirlpool method with heat and exercise, although LLLT still has some possibility of producing some biological effects on tissue metabolism. Experimental approaches comparing conventional physical therapy with specific laser irradiation are needed before clinical trials and a definite conclusion in the future.

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